Optimization of Chromosome Sectioning and Karyotype Analysis of the Wheat Neimai 8

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Abstract

Within the wheat root tip as material, 8 different collecting time and hydrochloric acid solution of comparative material from time of root tip the influence degree of the chromosome, thus to explore the optimal method of chromosome. The results show that the time in the morning 9:30-11:00, the sampling interval, splinter cell, the total number of cells is more, the most easily observed chromosome. In the interval, the best time for 10:00 a.m., medium-term phase division index, the highest is 11.76%; Acid dissociation, will use the 1 mol/L HCl solution from 13 min under 60 ℃, disintegrate effect is best, chromosome dispersion degree is the highest.

Key words: Neimai 8; chromosome preparation; Optimization

1. Introduction

Wheat, belong to Gramineae (*Gramineae*), wheat (*Triticum*), is one of the earliest cultivation of crops in the world. After a long period of development, it has become the world's most widely distributed, the largest and one of the highest nutritional value of food crops. In the Sichuan region planting of wheat, wheat by Mianyang 26×92R178 hybrid breeding, the original code in 2938. Try to participate in Sichuan province in 2002, an average of 362.53 kilograms per mu, compared with Sichuan wheat yield was 38.98% higher than that of the 28, than usual Sichuan wheat yield was 23.31% higher than that of the 28. Provinces try on average 360.02 kg per mu in 2003, compared with Sichuan wheat yield was 9.62% higher than that of the 107, third in groupFig1. In november 2003, approved by crop variety approval committee in Sichuan province, and recommend for the provincial key popularized varieties. In May 2003, is applied for the national varieties protection. In 2003, the ministry of agriculture inspection, quality meet the national GB/T17320-1998 wheat varieties for quality, quality of gluten wheat standards, particularly strong disease

resistance, immune to stripe rust, immune to powdery mildew. The gluten wheat varieties for quality, high quality, high yield, disease resistance, to ensure efficient and safe production, optional for producing high quality noodle wheat varieties of raw material base, order production, widely available.

In spring wheat for 8, 180-185 days in the whole stages. Strain 77-88 cm tall, medium to high tillering ability, ear rectangle, long awns, white shell, grain long ovate, white, semi-rigid, thousand seed weight 47.11 grams. Identified, immune to stripe rust, immune to powdery mildew, in the sense of 3. Quality analysis, density 765.5 g/L, 13.8% protein content, wet gluten content is 33.3%, the subsidence value of 27.9 ml, dough stability time 3.25 minutes. Therefore, the study of the varieties in the production of wheat for Sichuan region to provide the reference data is of great significance.

Chromosomes (*Chromosome*) is in the cell nucleus carries the genetic information (gene), a genetic objects, easily by alkaline dye dyed dark, so called chromosomes (chromatin); Its essence is DNA nucleotides, is composed of nucleoprotein, can use in the nuclei of alkaline dyeing, linear body structure. Due to the chromosome is the carrier of genetic material - gene, so for plants, chromosome structure and behavior of drives plant reproduction and reproductive behavior[1]. And study chromosomal structure and behavior of the commonly used method is the method of chromosome.

In the study of plant chromosomes, the root tip meristematic tissue as the main material, because conveniently, meristematic zone is easy to identify, such as take root germination, are not affected by season, it was less than other materials[2]. At the same time, because young root tip cells thrive after germination, meristem, producing less interference factors, so the related research is generally use root as a material for chromosome [3]. Therefore, this study will choose the wheat root tip of 8 as the main material of the experiment, using chromosome method to explore. But because the materials in the system in the process of dyeing time, pretreatment time, pretreatment of drugs, acidolysis and so on the many kinds of factors on the chromosome structure and behavior of different degrees of influence, so it is necessary to explore the optimization of chromosome to understand the wheat chromosome 8 structure and behavior. At the same time, the probe of chromosome optimization method can provide reference basis for the subsequent in situ hybridization and so on, for the wheat 8 karyotype analysis provide better experimental methods, for the agronomist let the theoretical basis for the widely cultivated varieties.

2. Materials and methods

2.1 Material

2.1.1 Wheat 8 varieties tested

2.1.2 Drugs and reagents

Ice water, anhydrous ethanol, acetic acid, hydrochloric acid, such as improved carbolic acid magenta

2.1.3 Main instruments

Microscope, water bath pot

2.2 Method and step

2.2.1 Based

To select good wheat seeds in petri dishes, wet filter paper with distilled water at the bottom, put in 1-4 ℃ refrigerator cryogenic treatment for 48 hours, then dark 1-2 days. Stay long 1-2 cm, respectively in at 8:30 in the morning, at 9, 9:30, at 10:00, 10:30, 11 shearing seed was about 0.5- 1.0 cm.

2.2.2 Pretreatment

Will remove the root tip of grouped by time marked Ⅰ, Ⅱ, Ⅲ, Ⅳ, Ⅴ, Ⅵ, each group of about 10 root (Ⅳ take about a total of 50), into the ice water soak for 24 h.

2.2.3 Fixed

Into the new system will each group after pretreatment of the apex carlo Ⅰ fixed liquid[(glacial acetic acid)/V (methanol) : V = 3:1] in and fixed under 1 ~ 4 ℃ for 24 h, and then putting the material into 75% ethanol preservation condition for

1 ~ 4 ℃.

2.2.4 Acid solution

Choose Ⅳ fixed set of materials with distilled water rinse after put into 1 mol/L HCl, in 60℃ water bath pot water bath respectively 4 min, 7 min min, 10 min, 13, 16 min, respectively notes for 1, 2, 3, 4, and 5 groups.

2.2.5 Dyeing

Extracted from hydrolysate root tip, 2 ~ 3 times, rinse thoroughly with distilled water about 3 min each time. In the centre of the glass slide again, use the blade resection and root hair zone, elongation zone kept meristematic zones, namely the meristematic side 1 mm or so, chop and with improved carbolic acid fuchsin dyeing about 10 min.

2.2.6 Tablet

After staining with dissecting needle materials, sealed by the cover glass, using filter paper net dyeing liquid absorption, taps, and fixed cover glass and glass slide two fingers, knock gently with anatomical beard needle handle, and then put a layer of blotting paper on the cover glass, fixed glass slide with left hand, right hand thumb pressure on blotting paper, make the material dispersion. Tablet before, if the material dyeing too shallow, can use alcohol lamp heating tablet again a moment, but you can't make the dyeing liquid boiling.

2.2.7 Microscopy

Under the microscope, with 40×16 or 100×16 lens for microscopy, select a ropriating dispersed evenly, visible more chromosome and dyeing modest film [4], per 100 cells per unit, each material to choose four units, medium-term split phase in the statistics of a unit cell number. And observation of cell morphology, staining degree and the degree of dispersion. Chromosome morphological basis for Levan [5]methods such as classification.

3 Results and analysis

3.1 The influence of different time of chromosome preparation

Through the six periods (8:30, 9:00, 9:30 10:00, 10:30, 11), remove the chromosome, the materials which are microscopy, randomly selected from 4 × 100 cells, each material again to count to the cells of its medium-term phase respectively, contrast, it is concluded that the data in table 1. The table 1 shows that the wheat root tip 8 materials to 9:30 in the morning - 11:00 this time interval, the large number of dividing cells, the most easily observed chromosome. In this range, the best time for 10:00 a.m. and cell division at this time the most strong, most of the root tip meristematic zones divided the middle of the stage division index is the highest, reached 11.67%, and the most stable middle phase number, the most conducive to further research such as karyotype analysis. And outside this range, although also can observe chromosome, but the

less divided, and the middle phase also is less, if choose this period based producer,it is not conducive to further observation.

Table 1 Effect of different sampling time on chromosome metaphase cells

Group number	Sampling time	Metaphase mitotic index	The apical cell observation
Ⅰ	8:30	2.87%	A few divided chromosomes was observed
Ⅱ	9:00	5.25%	More divided, chromosomes was observed
Ⅲ	9:30	7.79%	More divided, metaphase chromosomes was observed
Ⅳ	10:00	11.67%	More is in the middle phase, clear metaphase chromosomes
Ⅴ	10:30	10.19%	More is in the middle phase, clear metaphase chromosomes
Ⅵ	11:00	9.25%	More is in the middle phase, clear metaphase chromosomes

3.2 The influence of different solution from the time of chromosome preparation

Obtained in selected at 10:00 a.m. based within the wheat root tip 8, divided into 5 groups, use 1 mol/L HCl solution under 60 ℃ for 5 groups respectively the apex from 4 min, 7 min, 10 min, 13min, 16 min after production, the cell staining degree, dispersion degree of microscopic observation, record in table 2. The table 2 shows that by using 1 mol/L HCl acid solution under 60 ℃, in the process of dissociation, if time is too short, less than 10 min, will lead to inadequate cell dissociation and dispersion degree is too low, after tabletting chromosome is not easy to spread out; If time is too long, more than 16 min, in pressure when cells are easy to be broken, and difficult to dyeing, cause chromosome form is not clear. By comparison, when the time control in 13 min, disintegrate effect is best, cell is neither easy to stick together, will not cause damage to cells and chromosome, chromosome staining effect thereby, best medium-term phase most clearly. Under different dissociation time, observed chromosome morphological structure and clarity as shown in the figure below.

Table 2 Effect of different dissociations time on chromosome metaphase cells

Group number	dissociations time	Cell shape, staining and dispersion degree
1	4	Cytoplasmic staining deep, overlapping cells, more dispersed
2	7	Cytoplasmic staining deep, cell dispersion degree is low
3	10	Cytoplasmic staining shallow, chromosome staining deep, cell dispersion degree is better
4	13	Cytoplasmic staining very shallow, middle phase is clear, high dispersion
5	16	Cell shape is slightly damaged, chromosome staining is very shallow, middle phase is not clear

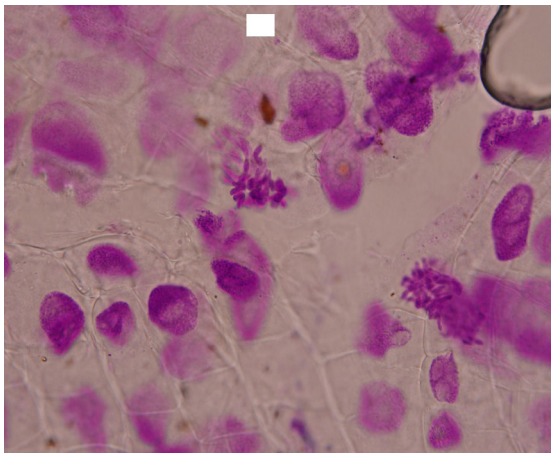

Fig 1. HCl dissociation 4min

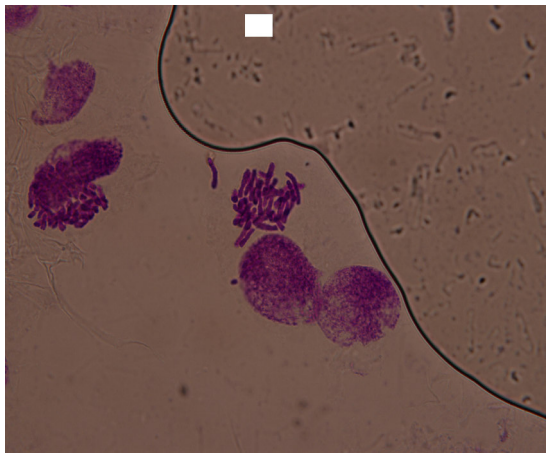

Fig 2. HCl dissociation 7min

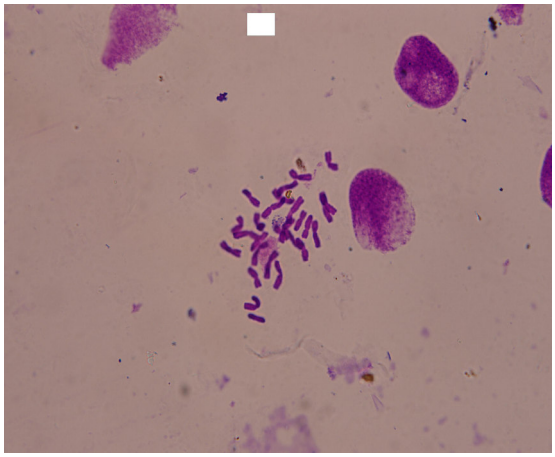

Fig 3. HCl dissociation 10min

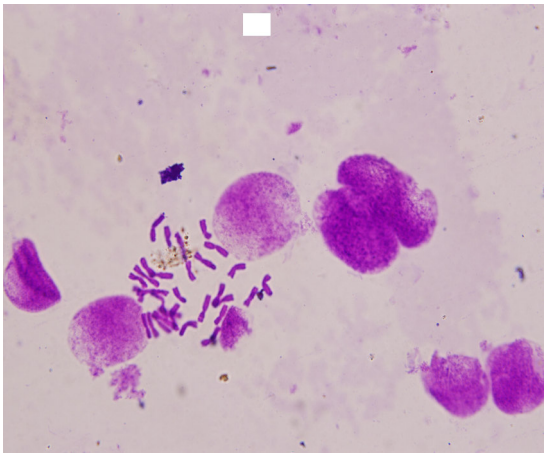

Fig 4. HCl dissociation 13min

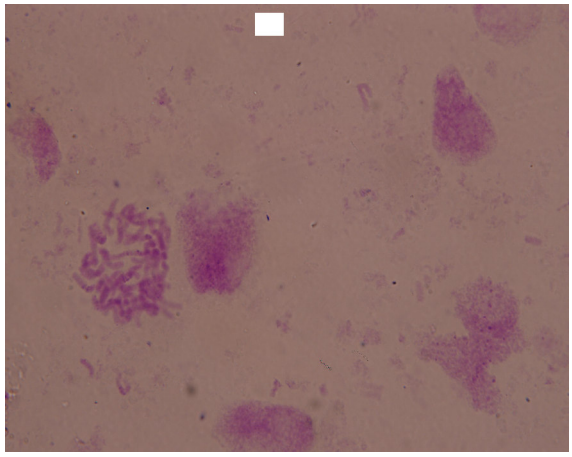

Fig 5. HCl dissociation 16min

4. Discussion

Chromosome technology is the common use of the plants karyotype analysis, for the wheat 8 dye structure and behavior research, in the actual operation, based on the time tend to be medium having a significant impact on the number of chromosomes, through domestic wheat chromosome 8 carries on the comparative analysis of different time shows that the time in the morning 9:30-11:00, best period the number of cells in the middle phase of more, and at this point in the middle of the chromosome is the most clear, observational study. Among them, the highest 10:00 a.m. based mid observed fragmentation index. Compared to this time, other split time out in the middle of less than, and chromosome is fuzzy, is not conducive to further karyotype analysis. Therefore, further study on the wheat chromosome 8, the producer should choose as far as possible when the time period - 11:00 at 9:30 am, the best time for 10:00.

In the dyeing system, the role of dissociative mainly remove pectin layer between cells and softens the cell walls, allowing for the tablet, makes the cells spread out and easy to count and observe. Dyeing system in general use of dissociative fluid is a concentration of 45% CH3COOH or 1 mol/L HCl, but as a result of CH3COOH weak acid, so we need the dissociation of for a long time, generally about 5 h. Compared with CH3COOH, HCl acid is stronger, therefore greatly shorten the understanding from the time, generally less than 30 min, and has better effect to disintegrate, cell dispersion degree is higher. But as a result of HCl acid is stronger, so the experiment must be rinsed with distilled water after fulfilling the dissociative material 2 ~ 3 times every time 3 min, and then on to the next step of dyeing.

For the wheat root tip cells disintegrate, 8 of this experiment using 1 mol/L HCl under 60 ℃ water bath, but time is a key water bath. In dissociation, if time is too short, inadequate cell dissociation and dispersion degree is not enough, after tabletting chromosome is not easy to spread out; if time is too long, then the pressure cells were easily crushed, and difficult to dyeing, unfavorable and microscopy, chromosomes at the same time easy to be destroyed, resulting in the form is not clear. By analyzing, the dissociation time of 13 min, the effect is best, cell dispersion degree good, dyeing and the color of deep, dark red or purple, cytoplasm colorless or only very shallow red, chromosomes are clearly visible [6].

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